

Effect of Diet and Mating on Oviposition in the Twospotted Stink Bug *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae)

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ABSTRACT Control females of the twospotted stink bug, *Perillus bioculatus* (F.), and those given an artificial diet mated repeatedly over their lifetimes when held with males. The average number of observed matings was 4 and 8.4 in the controls and those given the artificial diet, respectively. Mating and an adequate diet are prerequisites for laying a full complement of eggs in this insect. Virgins laid an average of 22 eggs compared with 138 in the multiply mated controls, 84 in singly mated females and 42 in multiply mated females given an artificial diet. The number of clutches deposited was not significantly affected by treatment, but the number of eggs per clutch was significantly lower in virgins and those given the artificial diet than in the controls or once mated females. The number of eggs per clutch showed no significant changes as the female aged. Oviposition rates were calculated over 5-d periods for each female and presented as eggs per day. In all experimental groups the number of eggs deposited per interval decreased as the females aged. By 27.5 d after the start of oviposition, females from all treatments laid one or fewer eggs per day. The decrease in eggs per day deposited by females as they aged is attributed to an increase in the time between clutch deposition. Egg viability showed no significant differences among the multiply mated controls, once mated females, or the multiply mated females given the artificial diet. This suggests that multiple mating is not required to maintain egg viability, but is necessary to maintain oviposition over the lifetime of the female. Low numbers of eggs oviposited by females given the artificial diet is attributed to the production of fewer mature follicles than in the controls and not to an impairment of the process of oviposition. In contrast, the lower number of eggs laid by virgin females results from an impairment of the oviposition process and not the impairment of ovarian follicle maturation.

KEY WORDS *Perillus bioculatus*, fecundity, artificial diet, oviposition rate, mating

Perillus bioculatus (F.), the twospotted stink bug, is a native North American predator (Knight 1952) of the Colorado potato beetle, a major defoliator of potatoes worldwide (Hare 1990). *P. bioculatus* has been shown to control Colorado potato beetle effectively in augmentative biological control programs (Biever and Chauvin 1992, Hough-Goldstein et al. 1996). However, economically feasible augmentative releases of *P. bioculatus* require a source of low cost, high quality insects (Glenister 1997, Hough-Goldstein and Whalen 1993). Two means of achieving this goal are to increase fecundity in mass-rearing programs and to improve the quality of artificial diets. Predatory stink bugs reared on artificial diets have lower fecundity than those reared on prey (De Clercq and Degheele 1992, Rojas et al. 2000; J. L. Wittmeyer, unpublished data), but the reason for this lowered fecundity is unknown and may involve diet quality, impairment of mating, or absence of the endocrine cascade required for oogenesis.

In some Hemiptera, mating influences oviposition (Ridley 1988, Adams 1999). Ovarian maturation of the first clutch of eggs in *P. bioculatus* is independent of female mating status, and by 9.5 d after emergence each of the seven ovarioles per ovary contains at least

one mature follicle in virgins or multiply mated females (Adams 2000). However, the effect of mating on oviposition has not been evaluated for *P. bioculatus*. Here I report the effects of female mating, age, and diet on *P. bioculatus* oviposition rates over their lifetimes to determine possible reasons for lowered fecundity in those given the artificial diet. A quantitative method for analyzing ovipositional data is presented and factors affecting oviposition are discussed.

Materials and Methods

Rearing. The *P. bioculatus* colony was reared on *Heliothis virescens* (F.) larvae using previously described methods (Adams 2000). The colony was obtained from eggs provided by Don C. Vacek (USDA APHIS PPQ MPFC, Mission, TX, 78573-2140) who reared them on larvae of another noctuid, *Spodoptera exigua* (Hübner). All rearing and experiments were conducted in a walk-in incubator held at $24.5 \pm 0.5^\circ\text{C}$ and 65% RH illuminated with high pressure 400 W sodium lamps (#47-1481, Hummert, Earth City, MO) from 0800 to 2200 hours. Twilight conditions were provided with two Sylvania Life Line F48-T12-

CW-HO fluorescent bulbs per bank in two banks from 0700 to 0800 and 2200 to 2300 hours.

Diets. The control diet consisted of previously frozen fifth-instar *H. virescens* larvae and insects given this diet are referred to as "prey-fed." A liver-based artificial diet developed by Tom Forester (USDA APHIS PPQ MPPC, Mission TX 78573-2140) was placed into food packets as in previous studies (Adams 2000). All experimental insects were reared on the control diet during their nymphal instars and were given either the control or artificial diet as adults.

Experiments. Newly emerged adults were collected at 0900 hours daily and placed in cages made from clear plastic wide-mouth jars (10 by 8.5 by 9.5 cm, Consolidated Plastics, Twinsburg, OH), with a 3-210/36 Nitex screen (Tetko, Lancaster, NY) covering a 6.5-cm-diameter hole in the cap. Each day the adults were provided with fresh diet and given a moist cotton dental wick. Eggs were collected daily, the number of clutches counted, and eggs per clutch determined. The eggs were placed in a clear plastic cup (#410, Fill-Rite, Newark, NJ), the cups were capped, and viability was determined. Observations were made over the lifetime of the females.

Three different mating regimens were evaluated on prey-fed insects for effects on oviposition: (1) Control females were held with males, 1-pair per cage, from emergence to evaluate the effects of multiple mating. (2) A 7- or 8-d-old female was held with a male of the same age in a clear plastic cage and allowed to mate. A higher percentage of insects at these ages mated than younger ones (Adams 2000). The times for coupling and uncoupling were recorded. At the termination of mating, these once-mated females were removed and held separately in cages without males. (3) Females were maintained as virgins from the time of emergence.

Another set of experiments compared the effects of the prey-diet with the artificial diet, provided to adults after emergence, on the number of eggs laid by females held with males. Matings were also recorded in each cage at the time of egg collection.

Data were collected from each female over its lifetime to determine the total number of eggs laid, the viability of the eggs, the number of clutches deposited, and the number of eggs per clutch. The ages at the start of oviposition and death were also determined. Daily observations were used to calculate eggs per day, eggs per clutch per day and egg viability per day to monitor the effects of aging.

Data Analysis. Data were analyzed using the general linear models procedure (SAS Institute 1987). The main effects in the model for total eggs deposited, number of clutches, eggs per clutch, percent hatch (arcsine converted percents), longevity, and age at oviposition were treatments and replicates. Each treatment was analyzed separately to determine the effect of aging or number of clutches deposited on egg viability, eggs per clutch and the number of eggs deposited. Main effects in the analysis of variance (ANOVA) were considered significant if $P \leq 0.01$. If the main effect was significant the means were sepa-

rated with the Student-Newman-Keuls multiple range test at the $P \leq 0.05$ level.

Eggs were collected daily, but the number of eggs oviposited was summed over a 5-d interval from the start of oviposition and the number of eggs laid per female per day during the interval was calculated for each female during her lifetime. This statistic smooths the daily oviposition peaks and valleys allowing the data to be analyzed with regression procedures (Motulsky 1999).

The percent of females that oviposited was analyzed with chi-square goodness-of-fit tests comparing each treatment with the control (Li 1957). Means in the text and table are followed by the standard deviation.

Results

Fecundity and Factors Influencing Fecundity. Repeated mating was observed in the pairs given either the control or artificial diet. There were 4.1 ± 5.4 (7 females) and 8.4 ± 9.4 (10 females) observed matings with ranges of 0-16 and 0-26 in control females and those given the artificial diet, respectively. Thus, females held with males tended to mate repeatedly over their lifetimes.

The number of females that oviposited was affected by treatment (Table 1). All controls and 81% of the once mated females oviposited, but 56% of virgins and 44% of females given the artificial diet oviposited. Multiply mated controls produced 138 eggs, but in the other treatments females oviposited significantly fewer eggs. The number of clutches produced by females over their lifetimes showed a low level of significance with treatment ($P \leq 0.025$) that varied from 12.7 clutches in those given the artificial diet to 5.6 clutches in virgins. However, clutch size was affected by treatment with the controls and once mated females producing significantly larger clutches than virgins or those given the artificial diet. The number of eggs per clutch was independent of female age ($P \leq 0.542$ at 43 and 183 df) and the number of clutches laid ($P \leq 0.481$ at 18 and 183 df). Thus, the treatments had little or no effect on the number of times a female oviposited, but did affect clutch size.

Virgins laid no viable eggs and viability in the remaining treatments showed no significant differences. Viability in these groups varied from 58 to 71%. Furthermore, egg viability was independent of female age and the number of clutches produced in controls ($P \leq 0.769$ at 33 and 58 df; $P \leq 0.800$ at 16 and 58 df), once mated females ($P \leq 0.0325$ at 29 and 18 df; $P \leq 0.050$ at 16 and 18 df) and those given the artificial diet ($P \leq 0.1418$ at 29 and 3 df; $P \leq 0.135$ at 15 and 3 df). Thus, multiple mating was not required for egg viability and female age had no apparent effect on hatch.

The preovipositional period was not significantly affected by treatment and varied from 8.6 d in controls to 12.1 d for virgins and those fed the artificial diet. Females that were permitted to mate once oviposited within 2.2 ± 1.6 d (13 females) of mating. Female longevity was not influenced significantly by any of

Table 1. Effect of mating and diet on fecundity and factors affecting fecundity in *P. bioculatus*

Parameter	Control (n)	Virgins (n)	1× Mated (n)	Art. diet (n)
%Oviposit ^a	100 (20)	56.2 (16)*	81.2 (15)	43.8 (14)*
Total eggs ^b	138.5 ± 65.4 ^a	22.0 ± 29.8 ^c	82.4 ± 78.6 ^b	45.8 ± 62.9 ^{bc}
Clutches ^c	10.6 ± 4.3 ^a	5.6 ± 3.2 ^a	7.3 ± 5.3 ^a	12.7 ± 5.2 ^a
Eggs/clutch ^d	13.6 ± 6.4 ^a	6.9 ± 4.3 ^b	12.8 ± 6.4 ^a	8.7 ± 4.9 ^b
%Hatch ^e	63.9 ± 20.6 ^a	0.0 ± 0.0 ^b	58.1 ± 26.4 ^a	70.7 ± 14.5 ^a
Pre-ovip ^f	8.6 ± 1.3 ^a	12.1 ± 2.9 ^a	[2.2 ± 1.6]	12.1 ± 5.4 ^a
Longevity ^g	41.2 ± 13.7 ^a	34.5 ± 16.5 ^a	35.1 ± 11.4 ^a	33.8 ± 26.4 ^a

Means are followed by their standard deviations. Values not followed by the same superscript letter are significantly different from each other at $P \leq 0.05$ as determined by Student-Newman-Keuls. The number in parentheses is the number of females observed over their lifetimes. Treatments consisted of prey-fed controls held continuously as pairs. Prey-fed virgins had no contact with males. 1× Mated prey-fed females mated once and held for the remainder of the experiment without contact with males. Art. Diet, females were fed artificial diet and held with males throughout the experiment.

^a Percentage of females that oviposited. Those percents followed by an asterisk are significantly different from the controls at $P \leq 0.05$ as determined by chi-square.

^b Average number of total eggs laid by insects over their lifetime and includes 0 values for those that did not oviposit. Treatment $P \leq 0.0001$ at 3 and 42 df.

^c Average number of clutches laid by a female over her lifetime. This value is based on the females that oviposited. Treatment $P \leq 0.0253$ at 3 and 25 df.

^d Average number of eggs per clutch based on 177 clutches for controls, 46 clutches for virgins, 88 clutches for singly mated females and 75 clutches for those given the artificial diet. Treatment $P \leq 0.0001$ at 3 and 183 df.

^e Percent hatch is the average percentage of eggs that hatched/female/treatment. ANOVA conducted on arcsine converted percentages. Treatment $P \leq 0.0001$ at 3 and 25 df.

^f Preoviposition period in days. Treatment $P \leq 0.028$ at 2 and 13 df. The value in brackets from once mated females is the time from mating to oviposition.

^g Longevity in days. Treatment $P \leq 0.0277$ at 3 and 25 df.

the treatments and varied from 35 d in the once-mated females to 41 d in the controls.

Oviposition Patterns and Rates. *P. bioculatus* females do not deposit eggs at well-defined intervals (Fig. 1). This control female oviposited the first clutch at 8.5 d and then produced a clutch per day for the next 4 d. After this time the oviposition pattern became increasingly more variable with fewer clutches deposited as the female aged. Because of this variability in oviposition patterns, raw data could not be used to analyze oviposition rates between the different treatments. However, when the number of eggs produced over 5-d periods was calculated the rate could be determined for each 5-d period over the lifetime of the female (Fig. 2).

In all treatments the slopes for the regression lines were negative, showing that the number of eggs ovi-

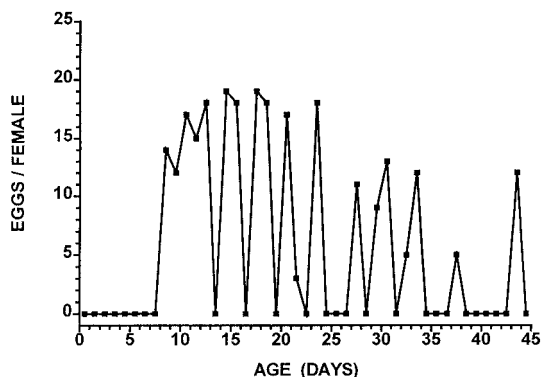


Fig. 1. Ovipositional pattern of a single control *P. bioculatus* female showing egg production per day over its lifetime.

posited per interval decreased with female age. After 27.5 d the rate of oviposition leveled off in controls (Fig. 2A), once mated females (Fig. 2B), and those given the artificial diet (Fig. 2D), but not in virgin females (Fig. 2C). Control females oviposited 10.1 ± 0.52 eggs per day at the beginning of the ovipositional interval, which then decreased at a rate of 0.33 ± 0.03 eggs per day to 27.5 d. After this time the slope of the line was not significantly different from 0 and females produced an average of 0.8 ± 2.3 ($n = 50$) eggs per day. Once mated females oviposited 7.83 ± 0.85 eggs per day at the beginning of the ovipositional interval, which then decreased at a rate of 0.27 ± 0.05 eggs per day to 27.5 d. After this time the slope of the line was not significantly different from 0 and females produced an average of 0.7 ± 1.0 ($n = 18$) eggs per day. The rate of decrease in once mated females was not significantly different from the control rate, but the number of eggs produced at the start of the interval (y intercept) was significantly lower. Virgin females oviposited 2.50 ± 0.36 eggs per day at the beginning of the ovipositional interval which then decreased at a rate of 0.07 ± 0.02 eggs per day to 37 d. The rate of decrease in virgins was significantly different from the control rate and the number of eggs produced at the start of the interval was 75% lower than in controls. Thus, mating has a significant affect on the rate of oviposition.

Females held with males and given the artificial diet oviposited 5.95 ± 0.71 eggs per day at the beginning of the ovipositional interval which then decreased at a rate of 0.20 ± 0.04 eggs per day to 27.5 d. After this time the slope of the line was not significantly different from 0 and females produced an average of 0.23 ± 0.62 ($n = 44$) eggs per day. The rate of decrease in once

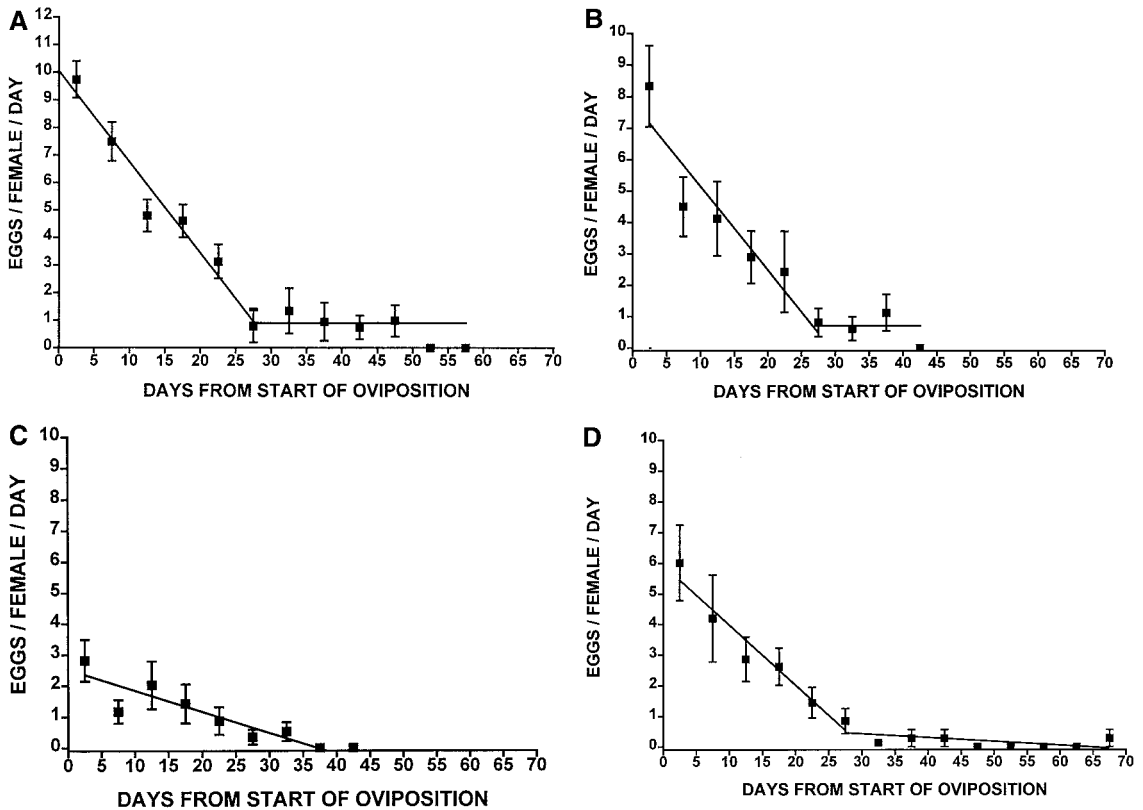


Fig. 2. Mean number of eggs produced by *P. bioculatus* females per 5-d intervals over their lifetimes. (A) Control females held with males and given prey produced a regression line from 2.5 to 27.5 d with a correlation coefficient, r , of 0.71, that was based on 112 points obtained from 20 females. The Y intercept is 10.1 ± 0.52 eggs per female per day with a slope of -0.33 ± 0.003 eggs per female per day that is significantly different from 0 at $P \leq 0.0001$. Data from 27.5 to 57.5 d are based on 50 observations from 16 females and the slope is not significantly different from 0. (B) Once mated females given prey produced a regression line from 2.5 to 27.5 d with a correlation coefficient, r , of 0.55, that was based on 56 points obtained from 12 females. The Y intercept is 7.83 ± 0.85 eggs per female per day with a slope of -0.27 ± 0.005 eggs per female per day that is significantly different from 0 at $P \leq 0.0001$. Data from 27.5 to 57.5 d are based on 18 observations from seven females and the slope is not significantly different from 0. (C) Virgin females given prey produced a regression line from 2.5 to 42.5 d with a correlation coefficient, r , of 0.48, that was based on 62 points obtained from nine females. The Y intercept is 2.50 ± 0.36 eggs per female per day with a slope of -0.07 ± 0.002 eggs per female per day that is significantly different from 0 at $P \leq 0.0001$. (D) Females held with males and given the artificial diet produced a regression line from 2.5 to 27.5 d with a correlation coefficient, r , of 0.60, that was based on 42 points obtained from seven females. The Y intercept is 5.95 ± 0.71 eggs per female per day with a slope of -0.20 ± 0.004 eggs per female per day that is significantly different from 0 at $P \leq 0.0001$. Data from 27.5 to 57.5 d, based on 48 points from seven females, has a slope not significantly different from 0.

mated females was not significantly different from the control rate, but the number of eggs produced at the start of the interval was 22% lower.

Discussion

Two conditions affecting oviposition in Heteroptera are the availability of mature ovarian follicles in the ovarioles and the ability to oviposit. To meet the first condition, an endocrine cascade must occur resulting in vitellogenin synthesis and subsequent uptake by the oocyte to form mature follicles (Davey 1993; Adams 1997, 1999). Currently, nothing is known about the endocrine cascade in any predaceous pentatomid. The second condition involves the ability to oviposit eggs contained in the ovarioles and requires

that females *P. bioculatus* are mated (Table 1). The percent of *P. bioculatus* females that oviposited was highest in multiply mated females and lowest in virgins. More eggs were oviposited with higher initial rates of oviposition in controls than in virgins or singly mated females, suggesting that multiple matings are required for the deposition of a full complement of eggs in prey-fed *P. bioculatus* (Table 1; Fig. 2). Furthermore, virgin females had similar ovarian scores to the controls (Adams 2000), showing that mature eggs were available for oviposition but were retained. Mating and oviposition are positively correlated in species from many orders of insects (Ridley 1988). Mated *Rhodnius prolixus* Stål females oviposit when mature eggs are present in the ovarioles, whereas virgins tend to retain their eggs (Davey 1965, 1967; Wang and

Davey 1993). Singly mated female *Riptortus clavatus* (Thunberg), an alydid, produced 40% fewer eggs than those that were multiply mated and held continuously with males (Sakurai 1996). *Thyanta pallidovirens* (Stål), a pentatomid, produced 33% fewer eggs when singly mated than did the multiply mated controls (Wang and Millar 1997). Baker and Lambdin (1985) showed no significant differences in the number of eggs laid by *Podisus maculiventris* (Say) virgins and those held with males, but De Clercq and Degheele (1997) showed about a 50% reduction in the number of eggs laid by virgins when compared with mated females. *P. maculiventris* also developed eggs in virgins with higher scores in virgins than in controls (De Clercq and Degheele 1997). Research in Ken Davey's laboratory showed that oviposition in *R. prolixus* is induced by the release of a myotropin that requires the presence of a spermathecal factor from mated females and ecdysteroid from mature ovaries (Adams 1999). Thus, maturation of ovarian follicles in *P. bioculatus* and these other Heteroptera is independent of mating, but oviposition is modulated by mating, suggesting that the *Rhodnius* model involving the interaction of mating with myotropin release might apply to many heteropteran species.

Egg viability showed no significant differences between the controls held with males, once mated females, or those given the artificial diet and held with males. Furthermore, egg viability showed no significant changes with female age or the number of clutches oviposited. Thus, a single mating appears to provide sufficient sperm to fertilize eggs over the lifetime of the female. This suggests that multiple mating is not required for the maintenance of egg viability, but is necessary for oviposition.

The number of eggs oviposited decreased with age in all *P. bioculatus* treatment groups (Fig. 2). This is not caused by a decrease in the number of eggs per clutch with age (Table 1), but to an increase in time between successive clutch deposition (Fig. 1). Other predaceous Heteroptera showed decreases in the number of eggs oviposited with age: *Orius sauteri* (Poppus) and *O. tantillus* (Motschulsky) (Nakashima and Hirobe 1999); *P. maculiventris* (Legaspi and Legaspi 1998); *P. sagitta* (F.) (De Clercq and Degheele 1992); *P. nigripinus* (Dallas) (Torres et al. 1998), and *R. clavatus* (Sakurai 1998). A phytophagous Hemiptera, *T. pallidovirens*, showed a decrease in eggs deposited after the ninth clutch (Wang and Millar 1997). Decreasing egg production with age is probably indicative of senescence and may be attributed to a decrease in ovarian follicle production and maturation.

Perillus bioculatus adult females given the artificial diet had equivalent longevity, mated as frequently and had similar egg viability as the controls, but oviposited fewer eggs (Table 1; Fig. 2). Furthermore, those females given the artificial diet had lower ovarian scores, matured fewer eggs, and took longer to enter vitellogenesis than the controls (Adams 2000). *P. maculiventris* maintained on prey as nymphs and then given artificial diet as adults also oviposited fewer eggs (De Clercq et al. 1998; J. L. Wittmeyer, unpublished data)

and had lower ovary scores than the controls (J. L. Wittmeyer, unpublished data). In other studies, artificial-diet-fed Asopinae pentatomids oviposited fewer eggs than prey-fed controls (De Clercq and Degheele 1992, De Clercq et al. 1998, Rojas et al. 2000). These data suggest that artificial diets maintain nonreproductive functions in adults that are necessary for survival, but negatively impact the vitellogenic process, resulting in the maturation of fewer eggs. Furthermore these females appear to oviposit eggs that are present in the ovarioles. Thus, predaceous pentatomids fed on artificial diets do not have impaired ovipositional abilities, but oviposit fewer eggs because they mature fewer eggs than the controls. This indicates that artificial diets can be evaluated effectively by looking at first cycle oocyte development or vitellogenin levels instead of monitoring egg production over the lifetime of the female, thereby reducing the assay time considerably.

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